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(RESEARCH ARTICLE)



Gas chromatography and mass spectroscopy analysis and phytochemical characterization of *Aegle marmelos* (Bael) leaf, Stem and its screening of antimicrobial activity

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Abstract

Phytochemical screening tests were conducted for five plant species and found that extract contains a variety of phytochemicals like saponins, tannins, flavonoids, terpenoids, glycosides and reducing sugars and among which there is higher level of precipitation for phenol and flavonoids. As they are essential source of antimicrobial agents against pathogen, their extract was tested for its antimicrobial activity by well diffusion method using Nutrient agar against human pathogenic bacteria like *Staphylococcus aureus*, *Escherichia coli*. This study provided evidence to confirm the presence of various medicinally important bioactive compounds or phytochemicals that have got biological importance and it justifies their use in the traditional medicines for the treatment of different diseases and these findings suggest that the selected plant extracts possess antimicrobial properties that could be used for biological control of bacterial cultures and these bioactive compounds serve as a source of antimicrobial agents against human pathogens. Medicinal plants have bioactive compounds which are used for curing of various human diseases and also play an important role in healing. Phytochemicals have two categories i.e., primary and secondary constituents. The phytochemical analysis of the plants is very important commercially and has great interest in pharmaceutical companies for the production of new drugs for curing of various diseases. This GC-MS has been used as standard protocol for a foreign substance identification because of its use to identify the particular specific test results which indicate or identify the presence of that particular substance. The aqueous leaf extract was used to identify and the phytochemical analysis used to find out the phytochemical constituents present in the taken plants. Plant showed that the alkaloids, terpenoids, phenol and tannins, reducing sugar, saponin, proteins, anthocyanin, coumarin and glycosides were found to be present in the given plants. Gas chromatography with mass spectroscopy detection is the state of the art methods for detection and identification of unknown compounds, it is also not infallible and many compounds are difficult with their accuracy certainty.

Keywords: Gas chromatography and mass spectroscopy; Phytochemical analysis; *Aegle marmelos* leaf; Antimicrobial activity

1. Introduction

Gas Chromatography–Mass Spectrometry (GC-MS) is a hyphenated analytical technique that combines the separation properties of gas-liquid chromatography with the detection feature of mass spectrometry to identify different substances; GC is used to separate the volatile and thermally stable substitutes in a sample whereas GC-MS fragments

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the analyte to be identified on the basis of its mass [1]. The GC-MS included include drugs detection, fire investigation and identification of unknown samples, including that of materials sample obtained from planet mars during probe mission early. There are other applications which include the environment cleanup; GC-MS is also analysis the particles from a human body order to help link a criminal to crime. Law enforcement is used for detection of illegal narcotics and drug sniffing dogs. Sports anti –doping analysis used to test athletes urine sample for prohibited. Chemical warfare agent's detection is increased capability in homeland security and public health preparedness [2]. Food, beverage and perfume analysis are some naturally present in the raw materials and some forming during processing. Medicine is metabolic disease also known as inborn errors of metabolism are now detectable by newborn screening test [3].

Phytochemical are the chemical compound produced by plants, and it's generally to help them thrive competitors, pathogens. The plant some phytochemical have been used as poisons and other as traditional medicine. Phytochemical is generally used to identify the plants compound that are under research with effect on health and are defined as essential nutrient [4].

Phytochemical are chemical produced by plants through primary or secondary metabolism, they have biological activity in the plants host and plays a role growth or defense against competitors, pathogens. Phytochemical are regarded as research compound rather than essential nutrient because proof of their possible health effects has not been establish [5]. Phytochemical Under research can be classified into major categories such as carotenoids and polyphenols, which include phenolic acid, flavonoids and stilbenes, flavonoids can be further divided into groups based on their similar chemical structure such as anthocyanins, flavones, flavanones and is flavones and flavonols [6].

Phytochemists study phytochemical by first extracting and isolating compound from the origin plants, the challenging in that fields include isolating specific compounds and determining their structure, which are often complex and identifying what specific phytochemical is primarily responsible for any biological activity[7].

1.1. Function of phytochemicals

The phytochemical is included the most recognized compound which is essential nutrients which are naturally contained in plant and are required for normal physiological function. The phytochemical are known phytotoxin that are more toxic to human as well as animals, some phytochemicals are antinutrients that interfere with the absorption of maximum quantity of nutrients [8]. Non- digestible dietary fibers from plant food, often considered as phytochemical are the new generally regarded as nutrient groups having approved health claims for reducing the risk of some types of cancer [9].

Vegetables, grains, legumes and plant based beverages has long term of health benefits and also there are some other kind of benefits which included that is no evidence that taking dietary supplements of non- nutrients phytochemicals supplements are neither recommended by health authorities for improving health [10].

Phytochemicals in freshly harvested plants foods may be used in degraded by processing techniques, including cooking and the main cause of phytochemicals loss from cooking is thermal decomposition, in the case of carotenoids such as lycopene present in tomatoes, which is mainly stable increase in contents from cooking due to liberation from cellular membranes in the cooked foods, that cooked food processing can also free carotenoids and other phytochemicals from matrix, increasing dietary intake [11].

Plants are used medicinally in different countries, and they are the source of many potent and powerful drugs. Plants have been an important source of medicine with qualities for thousands of years. Mainly on traditional remedies such as herbs for their history, they have been used as popular folk medicines [12]. It has been shown that *in vitro* screening methods could provide the needed preliminary observations necessary to elect crude plant extracts with potentially useful properties for further chemical and pharmacological investigations [13].

The aim of the present study was to investigate total phenols and flavonoids content of 53 traditionally used medicinal plants of western region of India. The plants/plant parts were extracted by cold percolation method in acetone and methanol. Qualitative phyto chemical analysis was done for various phyto constituents like alkaloids, tannins, cardiac glycosides, steroids and saponins. Total phenol and flavonoid content was quantitatively estimated. Various medicinal properties have been attributed to natural herbs. Medicinal plants constitute the main source of new pharmaceuticals and healthcare products; the history of plants being used for medicinal purpose is probably as old as the history of mankind [14]. The use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs from these plants as well as from traditionally used folk medicine. Extraction and

characterization of several active phyto compounds from these green factories have given birth to some high activity profile drugs. The use of traditional medicine is widespread in India [15].

Phytochemical screening of plants has revealed the presence of numerous chemicals including alkaloids, tannins, flavonoids, steroids, glycosides and saponins etc. Secondary metabolites of plants serve as defense mechanisms against predation by many microorganisms, insects and herbivores. Normally free radicals of different forms are generated at a low level in cells to help in the modulation of several physiological functions and are quenched by an integrated antioxidant system in the body [16]. However, if free radicals are produced in excess amount they can be destructive leading to inflammation, ischemia, lung damage and other degenerative diseases. Free radical reactions, especially with participation of oxidative radicals, have been shown to be involved in many biological processes that cause damage to lipids, proteins, membranes and nucleic acids [17].

Plants are known to produce these chemicals to protect them. While recent research demonstrates that they can also play an important role in protecting humans against diseases. Even some of these plants are in use as traditional medicine for centuries now. Most phytochemical like flavonoid, carotenoids and polyphenols have anti-microbial activity and serve as a source of antimicrobial agents against pathogens [18]. In Indian system a large number of medicinal plants have been used for many centuries for treating various diseases. Medicinal plants have been as remedies for human diseases because of its chemical contents of therapeutic value. Most traditional medicines are developed from nature. Thus plants remain a major source of medicinal compounds. As of record around 20,000 plant species are in use for medicinal purposes across the globe and around 70 % of them are from Indian subcontinent [19].

1.2. Phytochemical constituents

The chemical composition of bael is highly complex, containing many nutrients and other biologically active compounds, the proportions of which many vary considerably between strains and even among plants within the same field. Furthermore [20], the quantity of many of these constituent is significantly affected by differing growing, harvesting, processing and storage conditions that are not yet well understood [21]. The nutritional and pharmacological properties of the whole herb in its natural form, as it has been traditionally used, used result from synergistic interaction of many different active phytochemicals [22].

2. Material and methods

Phytochemical analysis

2.1. Collection and processing of plant material

The plant material of *Aegle marmelos* was collected in the month of June 2019 from the local area of Bangalore, Karnataka, India. The selected plant part removed and then washed under running tap water to remove dirt. The plant sample was then oven dried at 60°C for few days and was crushed in to powder and stored in polythene bags for future use.



Figure 1 Plant sample leaf and stem

2.2. Preparation of plant extracts

2.2.1. Water extract

The water extraction was carried out using classical method where grinded leaves material of 3 gm weighed using an electronic balance and was crushed in 100 ml of sterile water. Then the mixture was boiled at 50-60 °C for 30 minutes on water bath and it was filtered through Whitman No.1 filter paper. Then filtrate was centrifuged at 2500 rpm for 15 minutes and filtrate was stored in sterile bottles at 5°C for further use.

2.2.2. Ethanol extract

Samples (3 gm) were extracted with 100 ml of 95% ethanol on water bath at 70°C for 2 hr. The extracted samples were centrifuged and the supernatant was transferred into 50 ml volumetric flask. The volume adjusted to 50 ml with 95% ethanol and the samples were stored at -4°C until analysis. All water and ethanol extracts were filtered before analysis.

2.3. Phytochemical analysis

Qualitative analysis of extract was carried out to determine the presence of various bioactive compounds using the standard qualitative procedure.

2.3.1. Test for alkaloids

To 0.5ml of sample and 0.5 ml of concentrated hydrochloric acid was added. Then few drops of Mayer's reagent were added, presence of green colour or white precipitate indicated the presence of alkaloids.

2.3.2. Test for trepenoids

To 0.5 ml of plant sample, 2ml of chloroform was added and then concentrated sulphuric acid was added gently, formation of red brown colour at the interface indicated the presence of trepenoids.

2.3.3. Test for phenols

To 1ml of plant sample and 2ml of distilled water was added then few drops of 10% ferric chloride was added, formation of blue or green colour indicated the presence of phenols.

2.3.4. Test for tannin

To 0.5ml of plant sample and 1ml of 5% ferric chloride was added, formation of dark blue or greenish black is indicated the presence of tannin.

2.3.5. Test for reducing sugar

To 1ml of plant sample and 1ml of Fehling's A, Fehling's B was added to the sample, formation of red colour indicate the presence of reducing sugar.

2.3.6. Test for saponins

To 0.5 ml of plant sample with 1ml of distilled water was added and shaken in a graduated cylinder for 15 min lengthwise, formation of 1cm layer of foam was indicated the presence of saponins.

2.3.7. Test for proteins

To 1 ml of plants sample were taken and then few drops of HNO₃ was added, formation of yellow colour indicates the presence of proteins.

2.3.8. Test for steroids

To 0.5ml of plant sample with 4% NaOH solution and few drops of 1% CuSO₄ solution were added, violet colour appears indicates the presence of steroids.

2.3.9. Test for anthocyanin

1ml of plant sample mixed with 1ml of chloroform and concentrated H_2SO_4 sidewise, a red colour presence at the lower chloroform layer indicates the presence of anthocyanin.

2.3.10. Test for coumarins

To 0.5 ml of plant sample with 0.5 ml of 10% NaOH was added, formation of the yellow colour indicates the presence of coumarins.

2.3.11. Test for leucoanthocyanin

5 ml of isoamyl alcohol was added to 5 ml of aqueous sample extract, upper layer appear red in colour which indicates the presence of leucoanthocyanin.

2.3.12. Test for glycosides

To 1 ml of plant sample with 2 ml of chloroform and 10% of ammonia solution was added, formation of pink colour indicates the presence of glycosides.

2.3.13. Test for carbohydrate

To 0.5 ml of plant sample with 1 ml of Molisch's reagent and few drops of concentrated sulphuric acid were added, presence of purple colour or reddish colour indicated the presence of carbohydrate.

3. GC-MS analysis

3.1. Preparation of extract

The plant material of *Aegle marmelos* was collected from wild area, leaf should kept in hot air oven few day for properly dried, after that the dried leaf is pulverized to powder using mechanical grinder, then the required amount plant powder of *Aegle marmelos* was weighed and transferred to flask, treated with methanol until the powder was fully immersed, incubated overnight and filtrate through Whatman filter paper along with sodium sulfate to remove the sediments and trace of water in the filter paper, before filtering, the filter paper along with sodium sulfate was wetted with alcohol. The filtrate is then concentrated to 1 ml by bubbling nitrogen gas into the solution. The extract contains both polar and non-polar components of the plant material and 2 ml of the sample of the solution was employed in GC-MS for analysis of different compounds.



Figure 2 GCMS plant extract preparation

4. Antimicrobial activity of plant extracts

Antimicrobial tests for the leaves stem and roots of the studied plants were carried out against the pure cultures of *pseudomonas* and *Escherichia coli*. Bacteria were cultured overnight at 37 °C for 72 hour. Nutrient agar (20 ml) was

dispensed into sterile universal bottles. These were then inoculated, mixed gently and poured into sterile petridishes. After setting a number 3-cup borer (6 mm) diameter was properly sterilized by flaming and used to make 3 uniform well in each petridishes. A drop of molten nutrient agar was used to seal the base of each cup. The wells were filled with 50 of the extract concentration of 100 g/ml and allow for diffuse (45 minutes). The plates were incubated at 37 °C for 24 hours for bacteria. The zone of inhibition for the extract /fractions that showed antimicrobial activity was measured with antibiotic zone in mm.

5. Results

5.1. Phytochemical characterization

Table 1 Phytochemical screening results

Test name	leaves	Stem
Alkaloids	+	+
Trepenoids	+	-
Phenol	-	-
Tannin	+	-
Reducing sugar	-	+
Saponin	+	+
Proteins	-	+
Steroids	+	+
Anthocyanin	-	-
Coumarin	+	-
Leucoanthocyanin	+	+
Glycosides	-	-
Carbohydrate	+	-

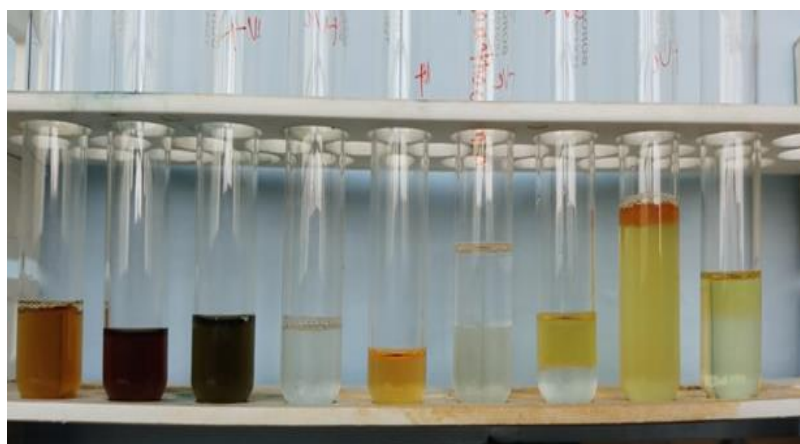


Figure 3 Phytochemical analysis results

5.2. GCMS analysis results

Table 2 GCMS analysis results

Sr. No	Name	R.T. (min:sec)	Similarity	Area %	Formula	Area	Exact Mass
1	Unknown 1	03:16.1	680	0.32	C ₆ H ₁₄ O ₅	12175118.00	166.08
2	Acetic acid, dichloro-, methyl ester	04:22.9	763	0.29	C ₃ H ₄ Cl ₂ O ₂	11133589.00	141.96
3	Benzene, 1,3-bis(1,1-dimethylethyl)-	04:54.1	932	0.20	C ₁₄ H ₂₂	7756004.00	190.17
4	11-Methyldodecanol	05:24.3	873	0.32	C ₁₃ H ₂₈ O	12164032.00	200.21
5	11-Methyldodecanol	05:28.5	864	0.35	C ₁₃ H ₂₈ O	13342022.00	200.21
6	Tridecane	06:07.7	961	0.14	C ₁₃ H ₂₈	5391644.00	184.22
7	1-Ethyl-4,4-dimethyl-2,5-dioximidazolidine	06:13.5	759	0.00	C ₇ H ₁₂ N ₂ O ₂	0.00	156.09
8	Decane, 2,3,5,8-tetramethyl-	06:29.1	942	0.02	C ₁₄ H ₃₀	833058.00	198.23
9	Heneicosane	06:34.4	904	0.24	C ₂₁ H ₄₄	9251481.00	296.34
10	Heptadecane, 2,3-dimethyl-	06:39.0	962	0.21	C ₁₉ H ₄₀	8203625.00	268.31
11	1-Octanol, 2-butyl-	06:42.4	807	0.03	C ₁₂ H ₂₆ O	1102525.00	186.20
12	Heptadecane, 2,3-dimethyl-	06:43.2	960	0.20	C ₁₉ H ₄₀	7780891.00	268.31
13	Heptadecane, 2,3-dimethyl-	06:48.1	950	0.22	C ₁₉ H ₄₀	8396649.00	268.31

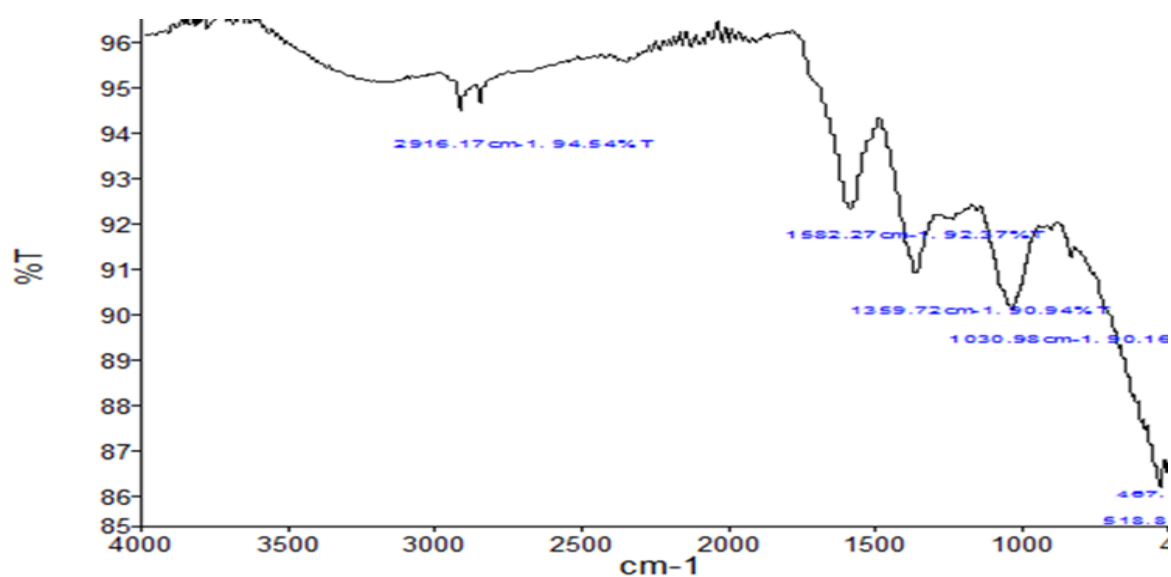


Figure 4 GCMS analysis graph

5.3. Antimicrobial activity test

Table 3 Antimicrobial activity tests

	Organism	Media	Extract	24 hours inhibition	48 hours inhibition	72 hours inhibition
Plants sample <i>Aegle marmelos</i>	<i>Pseudomonas</i>	Nutrient media	Stem	Present 3 mm	Present 2.4 mm	Present 4.1 mm
	<i>E. coli</i>	Nutrient media	Leaves	Present 2 mm	Present 4.2 mm	Present 2.5 mm

6. Conclusion

Aegle marmelos is a popular home remedy for many ailments such as wound, bronchitis, liver diseases, catarrhal fever, otalgia, lumbago, hiccup, ophthalmia, gastric disorders, genitourinary disorders, skin diseases, various forms of poisoning and psychosomatic stress disorders¹⁻². It has also aromatic, stomachic, carminative, demulcent, diaphoretic, diuretic, expectorant, alexiteric, vermifuge and febrifuge properties. Phytochemical screening leaves extracts of herbs *A. marmelos* one medicinally important tree had revealed the presence flavonoids, alkaloids, terpenoids, phenol and tanins saponins, steroids, coumarin Glycosides by positive reaction with the respective test reagent.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there are no conflicts of interest.

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